

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title: Characterization of the effects of putative sodium iodide symporter (NIS) inhibitors on endocrine and reproductive function in male and female rats.

LAPR Number: 20-10-001

Principal Investigator: Exemption 6

Author of this Document: Exemption 6 /RTP/USEPA/US

Date Originated: 01/26/2017

LAPR Expiration Date: 10/31/2020

Agenda Date: 11/01/2017

Date Approved: 11/13/2017

Date Closed:

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	11/13/2017	DMR	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	11/13/2017	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):

Characterization of the effects of putative sodium iodide symporter (NIS) inhibitors on endocrine and reproductive function in male and female rats.

Is this a continuing study with a previously approved LAPR?

No

2. Programmatic Information

a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.

CSS 16.01.01. High Throughput Toxicology (HTT), Task 2.1 (Development & optimization of in vitro assay to address EDC activity toward targets affecting thyroid hormones, Milestone 2, Implement NIS inhibition assay.

CSS 17.01 Adverse Outcome Pathway Discovery & Development (AOPDD), Task 1.3b, Development of AOPS for Reproduction in Vertebrates, Milestone 4, Development and implementation of an AOP linking changes in the hypothalamic-pituitary-adrenal axis to alterations in reproduction in the rat.

b. What is the Quality Assurance Project Plan (QAPP) covering this project?

IRP-NHEERL_RTP/TAD/ETB/Exemption 6/2015-001-r2, and QAPP-NHEERL/TAD/ETB/Exemption 6/2015-01-r0

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator Exemption 6	Phone Number Exemption 6	Division TAD	Mail Drop MD
	Lotus Notes Address Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	Branch ETB	

4. Alternate Contact:

Alternate Contact Exemption 6	Phone Number Exemption 6	Division TAD	Mail Drop MD
	Lotus Notes Address Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	Branch ETB	

SECTION A - Description of Project

1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

The U.S.EPA's Endocrine Disruptor Screening Program (EDSP) is required under U.S. Congressional mandates to screen and test pesticides, chemicals, and environmental contaminants to identify potential endocrine disruptors, determine adverse effects, dose-response, assess risk, and ultimately manage the risk under current laws. To more effectively manage the vast number of chemicals currently under the Agency's regulatory purview, the EDSP aims to incorporate the use of high-throughput (HTP) assays and computational models to initially screen and identify chemicals that have the potential to impact human health by disrupting the synthesis and/or function of hormones. This new approach will not only reduce the number of chemicals that require animal testing, but will also greatly accelerate the identification of chemicals, among thousands to be screened, that demonstrate a potential for disrupting specific targets within the endocrine system. In order to effectively use data from HTP assays for possible hazard identification and/or to prioritize chemicals for additional testing based upon data from HTP assays, it is necessary to demonstrate the predictive capability of the HTP approaches by comparing results with those observed in animal studies.

The purpose of the studies described in this LAPR is to obtain empirical data for metabolism, temporal and dose-responses for effects on endocrine and reproductive endpoints following exposure to environmental chemicals that have previously been identified as putative endocrine disruptors in high throughput (HTP) in vitro screening assays. These animal data are necessary to assess how well results from in vitro assays predict chemicals that can cause an adverse effect on endocrine and/or reproduction function in mammals. This step is critical for demonstrating the predictive capability of any given in vitro assay and to provide temporal and dose response data from animal studies that can be used to develop adverse outcome pathways (AOPs) and computational models that are appropriate for supporting the Office of Chemical Safety and Pollution Prevention's (OCSPP) decisions on chemical regulation and use in the USA.

Recently, our laboratory developed, validated, and implemented the use of a HTP in vitro screening approach to identify environmental chemicals that have the potential to disrupt the synthesis of thyroid hormones by inhibiting the uptake of iodide into the thyroid (Tox In Vitro, 2017, 40:68-78). The transport of iodide across the cell membrane into thyroid follicular cells is mediated by the sodium-iodide symporter (NIS), and its function is necessary to maintain circulating levels of thyroid hormones (i.e., thyroxine (T4) and triiodothyronine (T3)) that regulate an array of physiological processes that are essential for metabolism, cardiovascular function, reproductive function, and fertility, as well as fetal and post-natal neurodevelopment. Monovalent anions, such as the environmental contaminant perchlorate, are well documented competitive inhibitors of NIS, yet limited information exists for more structurally diverse chemicals. We applied the HTP approach to screen two large chemical libraries that contained approximately 1000 unique chemicals and covered a broad spectrum of chemicals that are currently under the U.S. EPA's regulatory purview. To facilitate prioritization of potential thyroid toxicants, a unique ranking system that incorporated cytotoxicity responses was developed to sort chemicals based upon NIS inhibition potency. Chemicals were ranked with a score that was relative to a known NIS inhibitor, sodium perchlorate. Top ranked chemicals were further tested in another in vitro assay using a rat thyroid follicular cell line to confirm inhibitory NIS activity. Using results from the HTP screening, in this LAPR, we have selected 6 chemicals to test in animal studies to evaluate the predictive capability of the HTP in vitro approach.

The objective of this LAPR is to conduct in vivo studies to evaluate the effects on thyroid hormone synthesis and reproductive function following exposure to select environmental chemicals that have been identified as putative inhibitors of sodium iodide symporter (NIS)-mediated uptake of iodide using a high-throughput (HTP) screening approach.

The animal studies proposed for this research are as follows:

- A short-term (4-days) exposure study using 21-day old (pre-pubertal) males and females will be used to evaluate temporal and dose-responses for endocrine and reproductive endpoints for select chemicals identified in HTP screening as potential actives.

-The Male and Female Rat Pubertal Assays will be used to evaluate a broader array of endocrine and reproductive endpoints following longer exposures (23-day exposures in females, 31-day exposures in males) to those chemicals that have been confirmed as active thyroid and/or reproductive toxicants during the short-term (4-days) study. These are existing protocols (see attachments) that are currently used to evaluate chemicals under the U.S.EPA's regulatory purview to identify those chemicals that disrupt the estrogen, androgen, or thyroid hormone pathways. Data from endpoints in these protocols are internationally accepted for regulatory decisions.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

The use of animals is necessary to accurately evaluate how well in vitro screening approaches identify groups of chemicals that can potentially disrupt the endocrine regulation of reproduction in mammals. It has been well established that the sole use of in vitro assays for hazard identification and risk assessment has limitations. Due to the complexity of the neuroendocrine regulation of reproductive function, some animal studies are still needed to understand the connections between chemical-induced cellular/biochemical changes in the brain, pituitary, and gonads that can lead to an adverse effect on reproductive function.

b. Justify the species requested:

The rat is the species of choice because there is a high degree of conservation between the human and the rat in the neuroendocrine regulation of puberty and reproductive function during adulthood, including the molecular pathways and targets of interest for this study. Furthermore, the rat is specified as an appropriate animal model for assays conducted under the U.S.EPA's Endocrine Disruptors Screening Program (EDSP). The rat is commonly utilized as a model species for hormonal and neuroendocrine system studies, therefore there is an abundant amount of literature that we can utilize as a valuable resource and potentially contribute towards.

3. How was it determined that this study is not unnecessary duplication?

We have searched the scientific literature extensively through PubMed and Google to determine that the proposed studies are not a duplicate of any other published reports using key words: thyroid toxicants, thyroid hormone disruptors, sodium iodide symporter (NIS), iodide uptake inhibitors, thyroxine, rats, perchlorate, etoxazole, oxyfluorfen, cyprodinil, niclosamide, methoxyfenozide, phenolphthalein.

We searched the U.S.EPA's ToxRef Database to determine no duplication of data that are directly relevant to the assessment of endocrine and reproductive endpoints evaluated in studies conducted under this LAPR.

Finally, these proposed animal studies will test chemicals that have only recently been identified as potential NIS inhibitors using a novel HTP in vitro assay for screening large chemical libraries. Our previous work represents the first large scale screening for NIS inhibitors among environmental chemicals. Thus, most of the chemicals selected for in vivo testing have limited or no previous information regarding endocrine or reproductive endpoints.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

The animal studies proposed for this research are as follows:

---- Short Term (4-days) Exposure Studies: These studies will use juvenile males and females to evaluate dose-responses for endocrine and reproductive endpoints of chemicals identified in HTP screening as potential actives.

Animals for this study will be received in-house on postnatal day (PND) 21 with testing beginning 5 days after arrival on PND 26.

Animals will be dosed daily by oral gavage for 4 days, with necropsy 2 hrs following the last dose. Endpoints measured include daily body weights, tissue weights, thyroid hormones and mRNA, and serum steroid hormones.

---- Male and Female Rat Pubertal Assays: Timed-pregnant dams will be received in-house on gestational day (GD) 14 and maintained until delivery of their pups naturally. All pups will be weaned on PND 21 and placed into respective dose groups (N=16).

Females will be weighed and dosed daily by oral gavage from PND 22 through PND 44 (e.g., 23 days of exposure). Beginning on PND 25, females will be monitored daily for vaginal opening (an indication of the onset of puberty). Beginning on the day of vaginal opening, daily vaginal smears will be collected and observed under a low-power light microscope for the presence of leukocytes, nucleated epithelial cells, or cornified epithelial cells to monitor the estrous cycle until necropsy. Females will be necropsied on PND 44 and tissue weights, thyroid hormones, and serum steroids will be measured.

Males will be weighed and dosed daily by oral gavage from PND 23 through PND 53 (e.g., 31 days of exposure). Beginning on PND 30, males will be monitored daily for preputial separation (e.g., an indication of the onset of male puberty). Males will be necropsied on PND 53, and tissue weights, thyroid hormones, and serum steroids will be measured.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

---- Short Term (4-days) Exposure Studies: The number of animals needed per dose group for these studies is based upon previous experiments in our laboratory and others that have documented the variation in hormone concentrations among rats. At least 3 dose groups and a vehicle control are needed to facilitate the use of U.S.EPA's recommended Benchmark Analysis for risk assessment. The inclusion of at least one additional positive control group is needed for quality assurance to demonstrate consistency of our results with those reported in literature for key molecular changes.

The positive control chemical, perchlorate, will be initially tested with 3 doses and 1 vehicle control group. N=10/ treatment group. Both males and females will be tested. Data from this study will be used to select a single dose for use in studies for the remaining 6 chemicals. Total rats needed for positive control test = 80

6 chemicals will be tested; Each with 3 dose groups, 1 vehicle control group, and 1 positive control group with N=10/treatment group. Total rats needed for 6 chemicals = (6 chemicals x 5 treatment groups x 10/group x 2 sexes) = 600 rats

Total rats needed for all short-term (4-day) exposure studies = 680 rats

---- Male and Female Rat Pubertal Assays: It is anticipated that the pubertal assays will only be used for a portion of the 6 chemicals. The number of animals needed for the pubertal studies is estimated for testing 4 chemicals that will be selected based upon the observation of strong thyroid/reproductive effects during the short-term study.

Each of the 4 chemicals will be tested with 3 dose levels, 1 vehicle control, and 1 positive control group for males and females.

The Agency's Test Guidelines for pubertal assays recommend N=15 per dose group. To maintain pair housing, the studies will use N=16 per dose group. Thus, 80 male and 80 female pups will be needed to test each chemical.

Theoretically, 16 dams (with litter size of 10 pups per litter) would yield 80 male and 80 female pups. However, to ensure sufficient numbers of males and females, will order 17 timed-pregnant dams per chemical.

Total number pregnant dams needed: (4 chemicals x 17 dams/chemical) = 68 dams

Total number of "offspring" needed: (16 pups/group x 5 treatment groups x 2 sexes x 4 chemicals) = 640 offspring

Total rats needed for all studies in this LAPR = Adults (680 short-term study + 68 dams)= 748 and Offspring = 640

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	748	640
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

- ☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

With the exception of the duration of exposure, treatments will be identical for both the short-term (4-days of exposure) and pubertal studies (23 or 31 days of exposure for females and males, respectively).

Experimental chemicals will be prepared in methyl cellulose, corn oil, or nano-pure water depending upon solubility. All doses will be administered by oral gavage (one dose per day between 7 - 9 AM), and dosing volume will be based on the daily body weight of each rat at 2 ml/kg. For chemicals with poor solubility, the dosing volume may be increased up to 5 ml/kg body weight.

Dosing solutions will be administered orally using steel feeding tubes. The appropriate gauge and length of the feeding tubes used will be based on the size (e.g., body weight) as recommended for rats in NHEERL-IACUC Rodent Gavage Best Practice (September 2017). Juvenile male and females rats (typical body weight, 50- 75 grams) will be dosed using 20 guage, 1 - 1.5 inch, curved needles, and older animals in the pubertal studies (typically, 76-300 grams) will be dosed with 18 -16 guage, 2 - 3 inch, curved needles.

Five treatment groups will include a vehicle control, a positive control, and 3 dose groups for each chemical. Doses will be selected based upon the oral LD50 for rats (e.g., not to exceed 10% of LD50) and available historical data (obtained from published literature or documented in the Agency's ToxRef Database).

The following chemicals have been selected (from approximately 1000 chemicals screened in our HTP sodium-iodide symporter (NIS) assay) as high priority for testing as in the short-term in vivo study, as indicated by ability to inhibit the NIS-mediated uptake of iodide.

Etoxazole, (CAS 153233-91-1)
Oxyfluorfen, (CAS 42874-03-3)
Cyprodinil, (CAS 121552-61-2)
Niclosamide, (CAS 50-67-7)
Methoxyfenozide, (CAS 161050-58-4)
Phenolphthalein (CAS 77-09-8)

Positive control chemical:
Ammonium Perchlorate (CAS 7790-98-9), or Sodium Perchlorate (CAS 7601-89-0)

b. Survival Blood Collections (method, volume, frequency):

n/a

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

Male and Female Rat Pubertal Assays: Beginning on PND 25, females will be monitored daily for vaginal opening. Beginning on the day of vaginal opening, estrous cyclicity will be monitored by the daily collection of vaginal smears until necropsy. Males will be monitored for preputial separation beginning on PND 30.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

n/a

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

n/a

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:

Animals will be identified per ear punch and assigned animal numbers made visible on the cage cards. Animals will be monitored daily and body weights will be recorded throughout the treatment period by **Exemption 6**

Exemption 6Exemption 6Exemption 6

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

n/a

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

n/a

c. Testing methods:

n/a

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

n/a

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

n/a

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

n/a

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

n/a

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

n/a

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

n/a

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of

sutures/staples, frequency and duration of monitoring including weekend and holiday care):

n/a

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

n/a

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

☐ Yes ☒ No

f. Identify any surgical procedures performed at other institutions or by vendors:

n/a

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

If any animals appear to be sick by displaying signs of systemic toxicity, appear to have increased stress, and/or are not eating, then they will be promptly removed from the study and immediately euthanized. The attending veterinarian will be consulted when appropriate to determine appropriate course of action.

Etoazole, ocyfluorfen, cyprodinil, niclosamide, and methoxyfenozide (all pesticides) and perchlorate (environmental contaminant) will be used at doses that are not expected to produce any symptoms of toxicity that would require humane intervention as based on previous dose response data reported in literature and to USEPA during chemical registration.

As a chemical listed under the Agency's TCSA inventory, phenolphthalein, does not have dose response data and no known symptoms of toxicity have been reported.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

Animals will be removed from the study if they show signs of excessive salivation, diarrhea, lethargy, and/or other signs of illness. Any animal that loses more than 20% of its body weight or shows any lasting signs of distress (excessive vocalization, porphyrin staining) or discomfort (hunched posture, stretching) will be removed from the study and receive immediate veterinary attention. Animals will be monitored and weighed on a daily basis during the dosing period by the PI or technical staff listed in section E1.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

n/a

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

- | | |
|---|-----|
| a. Animals to be purchased from a Vendor for this study: | 748 |
| b. Animals to be transferred from another LAPR:
LAPR Number that is the source of this transfer: | 0 |
| c. Animals to be transferred from another source: | 0 |

d. Offspring produced onsite (used for data collection and/or weaned): 640
e. TOTAL NUMBER of animals for duration of the LAPR 1388

2. Species (limited to one per LAPR): Rat(s)

3. Strain: Sprague-Dawley
Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

4. Sources of animals:
Charles River Laboratories

5. Provide room numbers where various procedures will be performed on animals:
Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

no

Room Numbers:

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)
n/a

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.
n/a

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

We may need assistance from animal care contract staff with dosing, body weight measurements, and vaginal lavage (smears). A memo will be sent to the ARPO requesting assistance during the pre-planning period for each study.

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

We request that polycarbonate cages with heat-treated pine shavings be used, where dams will be housed one per cage and weanlings will be housed two per cage. Our study will require that only pine shavings, with no nesting material or enrichments, be used to prevent potential endocrine disruptor exposure.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.

Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Test Agents	Maximum Dose	LD50(oral) rat
Etoxazole	500 mg/kg	5000mg/kg (oral)
Oxyfluorfen	500 mg/kg	5000mg/kg (oral)
Cyprodinil	500 mg/kg	5000mg/kg (oral)
Niclosamide	250 mg/kg	2500mg/kg (oral)
Methoxyfenozide	500 mg/kg	5000mg/kg (oral)
Phenolphthalein	250 mg/kg	>2500mg/kg (oral)
Ammonium Perchlorate,	420 mg/kg	4200mg/kg (oral)
Sodium Perchlorate	210 mg/kg	2100mg/kg (oral)

Nitrile gloves, safety glasses, face mask, and disposable labcoats will be worn when working with dosing solutions and animals.

All substances will be of highest grade available.

1% Methyl Cellulose (CAS

Corn oil will be food grade and used within one year of opening.

Phenolphthalein, oxyfluorfen, cyprodinil, ammonia perchlorate, and sodium perchlorate have been added to HSRP # 806, The evaluation of chemicals on the hypothalamic-pituitary-gonadal (HPG) axis and development.

Etoxazole, niclosamide, and methoxyfenozide do not require a HSRP.

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

Etoxazole, oxyfluorfen, cyprodinil, methoxyfenozide are registered pesticides and perchlorates are known environmental contaminants. Phenolphthalein is currently listed on the Agency's TCSA list, and used in industrial and laboratory applications as an indicator in acid-base titrations.

None are available in pharmaceutical grade.

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

n/a

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

Chemicals will be weighed and dosing solutions prepared in fume hood, and personnel will use personal protection equipment to include nitrile gloves, safety glasses, lab coats.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform

the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	Develop study and assist with organization or any animal handling necessary.	Over 35 years of animal handling and performing research studies for endocrine studies. Has completed all NHEERL-required training.
Exemption 6	Technical Staff	Develop and organize study, prepare doses, monitor animals and necropsy	Over 18 years of animal handling and research study execution. Has completed all NHEERL-required training.
Exemption 6	Technical Staff	Animal husbandry and assisting with dosing, animal monitoring, and necropsy	Over 6 years of animal handling/study execution. Has completed all NHEERL required training.
Exemption 6	Technical Staff	Develop and organize study, prepare doses, monitor animals, animal husbandry, vaginal smears, necropsy	Over 18 years of animal handling and conducting research studies. Has completed all NHEERL required training.
Exemption 6	Associate Principal Investigator	Animal husbandry, assist with dosing, vaginal smears, necropsy when other are unavailable	Over 25 years experience in animal handling, study design and execution. Has completed all NHEERL required training.
Exemption 6	Post-Doc	Develop and organize study, prepare doses, monitor animals, animal husbandry, dosing, vaginal smears, necropsy	Three years of animal handling and research study design and execution. Has completed all NHEERL required training
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year n/a

2. Breeding protocols and recordkeeping n/a

3. *Methods for monitoring genetic stability* n/a
4. *Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR* n/a

SECTION G - Euthanasia

1. *When will the animals be euthanized relative to experimental procedures?*

Since the dams that birth the pups for the pubertal studies will remain untreated, efforts will be made to offer the dams for transfer to the training colony or other appropriate LAPRs after their litters have been weaned.

Experimental animals will be euthanized at the completion of each study to measure endpoints as indicated in Section B1. Short-term exposure studies: PND 29; Pubertal Studies: approximately PND 53 for male rats and PND 44 for female rats

2. *Describe the euthanasia techniques:*

Method(s): Decapitation, without anesthesia

Agent(s):

Dose (mg/kg):

Volume:

Route:

Source(s) of information used to select the above agents/methods:

- 2013 AVMA Guidelines on Euthanasia / Professional experience

Regarding decapitation an alternate guillotine will be readily available when animals are to be sacrificed. All staff that perform this method of euthanasia are highly experienced with this procedure.

3. *Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).*

Decapitation without anesthesia is compliant with 2013 AVMA Guidelines for Euthanasia

4. *Describe how death is to be confirmed.*

Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Euthanized by Animal Care Contractor

Transferred to another study

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

☒ Yes ☐ No

SECTION I - Assurances

1. *Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.*

2. *All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.*

3. *Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.*
4. *The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.*
5. *The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.*
6. *All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.*
7. *I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.*
8. *The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.*

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	10/25/2017

Submitted: 10/25/2017

Resubmitted: 10/25/2017

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	Approval Date	Phone Number	Division	Mail Drop
Exemption 6	10/25/2017	Exemption 6 Lotus Notes Address Exemption 6 Exemption 6 Exemption 6 RTP/USEPA /US	TAD Branch ETB	MD Submitted to Branch Chief for Approval 10/25/2017 11:24 AM

ATTACHMENTS



20-10-001 PI resp1.pdf GavageBestPractice2017-09-28.docx EPA-HQ-OPPT-2009-0576-0009[1].pdf



EPA-HQ-OPPT-2009-0576-0010[1] male pubertal.pdf

Actions

First Update notification sent:

Second Update notification sent:

First 2nd Annual notification sent:

Second 2nd Annual notification sent:

1st Expiration notification sent:

2nd Expiration notification sent:

History Log: